Faculty of Resource Science and Technology

ANALYSES OF MICROBIAL GROWTH ON LOCALLY PRODUCED *RAGI* FOR *TAPE* FERMENTATION

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Analyses of Microbial Growth on Locally Produced Ragi for Tape Fermentation

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ABSTRACT

In this study, *ragi* samples obtained from different local suppliers were analysed for different microbial growth on the solid surface. For the preparation of *tapai*, cooked rice was inoculated with the powdered *ragi*, incubated at 27^{o}C and then isolates for the microbes exist every 12 hours. Identification of microbes was performed using visualizing of staining specimen with methylene blue under phase contrast microscope. The results obtained showed three fungal isolates (two moulds and one yeast) and one filamentous bacterium. The mould isolates were identified as *Mucor* sp. and *Rhizopus* sp. while the yeast isolates was identified as *Saccharomyces* sp.. The bacterium was identified as *Streptomyces* sp. that may be due to the contaminants. Within 24 hours, the production of glucose was more rapid in Maranek (121.2 g/L) with 65.68% increased compared to Tambirat (25.2 g/L) *tapai* with 48.41% increased while the concentration of ethanol was higher almost two times in Maranek (46.8 g/L) compared to Tambirat (24.2 g/L) *tapai* with 92.31% and 93.39% of increased in ethanol production respectively.

Key words: *ragi*, *tapai*, phase contrast microscope, moulds, yeast.

ABSTRAK

Dalam kajian ini, sampel ragi yang diperoleh daripada pembekal tempatan yang berbeza dianalis kehadiran pelbagai jenis mikrob di atas permukaan pepejalnya. Untuk persediaan tapai, nasi yang telah dimasak digaul bersama serbuk ragi, dieram pada suhu 27^{o}C dan diasingkan mikrobnya setiap 12 jam. Pengenalpastian mikrob dilakukan melalui pemerhatian spesimen yang telah diwarnakan menggunakan metilin biru di bawah mikroskop fasa kontras. Keputusan mendapati sejumlah tiga fungal dapat diasingkan (dua dari kulat dan satu dari yis) dan satu ialah bakteria berfilamen. Kulat yang diasingkan dikenalpasti sebagai Mucor sp. dan Rhizopus sp. Manakala yis yang diasingkan pula dikenalpasti sebagai Saccharomyces sp.. Bakteria yang wujud dikenalpasti sebagai Streptomyces sp. akibat daripada dicemari. Dalam 24 jam, penghasilan glukosa berlaku dengan lebih cepat dalam tapai Maranek (121.2 g/L) dengan kenaikan sebanyak 65.68% berbanding tapai Tambirat (25.2 g/L) dengan kenaikan sebanyak 48.41% manakala kepekatan etanol dalam tapai Maranek (46.8 g/L) adalah lebih tinggi hampir dua kali ganda berbanding Tambirat (24.2 g/L) dengan kenaikan penghasilan etanol sebanyak 92.31% and 93.39% masing-masing.

Kata kunci: ragi, tapai, mikroskop fasa kontras, kulat, yis.

LIST OF ABBREVIATION

g	gram
g/L	gram per litre
hr	hour
min	minute
mL	milliliter
mm	millimeter
NA	nutrient agar
NB	nutrient broth
rpm	revolution per min
^{o}C	degree celcius
µm	micrometer

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CHAPTER I

INTRODUCTION

Fermentation is one of the oldest and most economical means of producing and preserving foods. It is believed that fermentation of foods was originated in China for preservation of cereals and legumes (Vijayalakshmi *et al*., 1997). The practice has been extended to many other foods including vegetables, fruits, edible parts of plants, fish, milk and meats. According to Vijayalakshmi *et al*. (1997), fermented foods are prepared by microbial action on one or more components under relatively controlled conditions. It causes changes in their physical, biochemical and nutritional qualities.

Fermented food is very popular among the people in Asia-Pacific region. Since this region is characterized by its tropical and subtropical climate, it is very important to preserve the harvest crops. The inhabitants consumption varies according to geographic and climatic condition. Primarily, the inhabitants of the tropical Southeastern regions consume rice. Those in subtropical and temperate zones of the Northeastern region including Northern China, Korea and Japan consume wheat, buckwheat, barley, corn, millet and soybeans in addition to rice. The consumption of rice as a staple food has resulted in at typical food processing technology.

Food technology becomes more important discipline since the rapid increase in the world population. Fermentation of foods is a way of solution to provide the necessary quantities of foodstuffs instead of preservation of existing foods and maintenance or improvement of their quality. Fermented foods have numerous advantages over the raw materials from which they are made, not only in improved flavour and texture, appearance and aroma, but also increase in storage life of foods. The cost of preservation also rather cheap compare to the preservation by canning and freezing. Their dietary fiber content is relatively high and very desirable foods for infants, expectant and nursing mothers and invalids (Vijayalakshmi *et al*., 1997). The increased content of vitamins, proteins and energy in fermented foods also makes them valuable in preventing malnutrition.

The fermented food products can be divided into two categories; products that result from bacterial fermentation and products from fungal fermentation (Lee and Fujio, 1999). Products from bacterial fermentation include Japanese *natto* and Philippine *nata* while products resulting from fungal fermentation majority are *tapai* or *tapai*-like products. The fermented foods are produced by the local people in small scale. Formerly the preparation is passed from one generation to another generation, very often with improvement and addition of ingredients. But nowadays, fermented foods also being produced with improved equipment and using bioprocessing innovations for quality control (Kuswanto, 2008).

Tapai is a type of fermented rice, has sweet and sour alcoholic taste. It is made from various substrates such as cassava tubers, cooked rice or cooked glutinous rice (Gandjar, 2003) and mix with powdered *ragi*. *Ragi* acts as the fermentation starter and used in other countries but

under different names. In the China *ragi* is called *chu*, in Korea *nuruk*, in Japan *koji*, in Indonesia and Malaysia *ragi*, in Philippine *bubod*, in Thailand l*oopang* and in India *marchaa.* The term *ragi* will be used in this project. *Ragi* is made of rice flour and other ingredients (mostly spices) with the presence of mould and yeast through natural infections from the surroundings and from the equipment used by the manufacturer (Djien, 1972). The ingredient of *ragi* may vary according to the countries.

During fermentation of foods, complex materials such as carbohydrates, proteins and fats are metabolized to yield others such as ethanol, organic acids and pectic hydrolysates (Vijayalakshmi *et al*., 1997). The flavour of the foods may be formed through condensation of fatty acids and alcohols into esters and oxidation of fatty acids to carbonyl compounds (Rose, 1982; Reed, 1983; Wood and Hodge, 1985, cited from Spencer and Spencer, 1997). According to Cronk *et al.* (1977), Went and Geerligs (1895) were the first to isolate and identify the microorganisms that thought to be essential for the fermentation. From their discovery, at least one amylolytic filamentous fungus and one or more alcohol-producing yeast were present during the *tapai* fermentation. In *tapai* fermentation, the substrate becomes soft and some acid are formed as shown by the soft alcoholic smell produced. The fermentation requires microorganisms in order to convert the starch into glucose. Since there are spices added during the *ragi* preparation, it may contribute to the growth of other microorganisms or may inhibit development of undesirable microorganisms (Sie, 1962; Soedarsono, 1972; cited from Steinkraus, 1983).

Therefore the objective of this study is to analyse visually the different microbes exist on *ragi* and *tapai* hence giving rise to different degree of sweetness and flavours during *tapai* fermentation.

CHAPTER II

LITERATURE REVIEW

2.1 *Tapai* Fermentation

Tapai is a traditional fermented food. It is partially liquefied (Cronk *et al*., 1977), with a sweet-acid taste and mild alcoholic flavour (Djien, 1972). It is a popular delicacy especially during the traditional ceremonies or just consumed as snack item without further processing after the fermentation (Cronk *et al*., 1977). It is prepared among the traditional manufacturers using cassava, rice or glutinous rice as the substrates. The preparations were transferred from generation to generation. According to Gandjar (2003), a carbohydrate source and an inoculum containing the microorganism is necessary to prepare *tapai*. The inoculum is called *ragi* and the success of a good *tapai* depends on the preparation of substrate and *ragi* itself.

In Sarawak, *tapai* from cooked rice is popular among the local. The preparation of *tapai* may vary from one place to another place but usually common ingredients such as powdered *ragi* are added to the substrate. In the traditional method, the fermentation of *tapai* initiated by addition of powdered *ragi* while in modern method, the starter used consist of a single organism or a combination of organism produced by microbiological method (Steinkraus, 1983). According to local manufacturer from Kampung Maranek and Kampung Tambirat, the amount of *ragi* added is about four to five pieces per one kilogram of rice. Local

manufacturers usually used traditional method in producing *tapai* hence the quantity also in small scale. Powdered *ragi* is mixed with cool cooked rice before the mixture is wrapped and allowed to ferment at room temperature (25-30^{o}C) for one day and after that it can be consumed. For the purpose of wrapping, various types of wrapping **(Figure 1)** such as banana leaves, nipah leaves, cocoa leave and '*buan*' leaves were used (Based on interview session with the manufacturers from Kampung Maranek and Kampung Tambirat, 2007). A flow sheet of the process is given in **Figure 2.**

Figure 1. *Tapai* in various wrapping. *Tapai* wrapped with banana leaves (left) and *tapai* in nipah leaves (right).

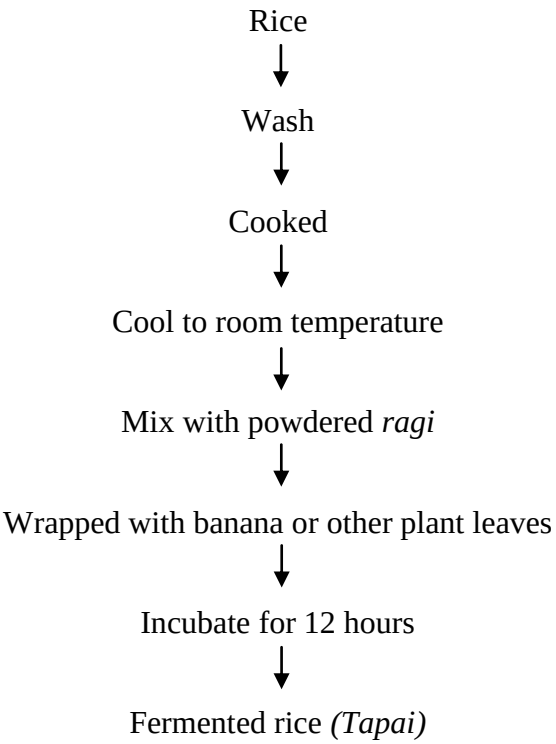

Figure 2. Flow sheet: Manufacture of traditional *tapai* from rice.

Tapai is a very perishable product since the fermentation continues even after the optimum stage of fermentation has been reached. Therefore it must be consumed immediately, but if it is chilled, it may be kept for about two weeks (Kuswanto, 2008). If *tapai* is allowed to ferment longer, it will result in greater liquefaction of the rice and is consumed as an alcoholic beverage, upon filtration.

2.2 Fermentation Starters: *Ragi*

Ragi is a dry circular cakes prepared locally from rice flour with distinctive spices (Cronk *et al*., 1977). It has many varieties of shapes and sizes; some spherical balls or round flattened cakes **(Figure 3)**. *Ragi* is used as one of the materials to make *tapai*. *Ragi* is produced using traditional method by household or village manufacturer using closely recipes and available in local market.

Figure 3. Locally produced *ragi*.

The production of *ragi* can be seen in the area of Asajaya and Kota Samarahan, Sarawak. The manufacturers still using traditional ingredients with a little adjustment in mixing the ingredients. Equipment such as a machine **(Figure 4)** was used to mix the ingredients and formed the dough.

Figure 4. Machine for mixing the mixture to form dough before flatten into cakes.

The production of *ragi* may vary from one place based on the ingredients that they used. Usually basic ingredient such as ginger, onion, garlic, white pepper, '*lengkuas'*, '*cekor'* **(Figure 5)**, '*akar kayu manis'* **(Figure 5)** and *'kulit kayu manis'* were used. Some addition spices such as *'das manis'* and *'das pedas'* **(Figure 6)** were used by the manufacturer from Kampung Tambirat. The starters are prepared under non-sterile conditions by the manufacturers (Based on the interview session with the manufacturers from Kampung Maranek and Kampung Tambirat).

Figure 5. '*Cekor*' (left) is added in preparation of *ragi* and moisten with boiled stock of '*kayu manis*' (right).

Figure 6. Spices ('*das manis*' and '*das pedas*') added in the preparation of *ragi*.

Ragi is prepared from rice flour that is mix with grinded spices as above. The mixture is moisten with the boil water that already been cold taken from *'akar kayu manis'* and *'kulit kayu manis'*. It is then mix to form a dough. The flattened cakes are incubated for three to four days at room temperature followed by drying under the sun to preserve the cakes. A flow sheet of the process is given in **Figure 7**.

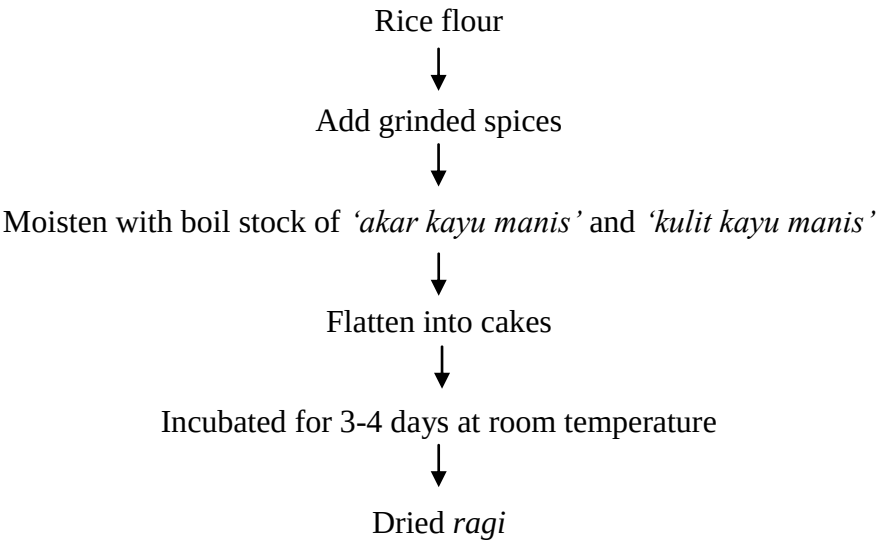

Figure 7. Flow sheet: *Ragi* preparation of local manufacturers (Based on the interview session with *ragi* manufacturers from Kampung Maranek and Kampung Tambirat, August 2007).

2.3 Microbial Growth

Essential microorganisms are preserved during the air- or sun-dried *ragi* cakes for several months at room temperature in the tropics. Since there are spices added during the *ragi* preparation, it may contribute to the growth of other microorganisms or may inhibit development of undesirable microorganisms (Sie, 1962; Soedarsono, 1972; cited from Steinkraus, 1983). Raw dry spices contain a wide variety of microorganisms and some inhibitory properties also exist due to the essential oils that can be found in spices. There were studies about the role of oriental herbs in the traditional starter preparation to their effect on the microflora of starter during the manufacture. It was observed that oriental herbs stimulate the growth of yeasts and moulds (Dung, 2004). Under certain condition for the

production of *ragi*, the growth of amylolytic yeasts and moulds may be favored while growth of undesirable bacteria is inhibited.

There are various studies done by the researchers about the fermentation starter in different countries including Thailand, Vietnam, Indonesia and Malaysia. Savitree *et al.* (2008) reported that in Thai traditional fermentation starter (Loog-pang), it comprised of *Saccharomycopsis fibuligera* which showed strong amylolytic activity. In addition, according to Lee and Fujio (1999), among the yeast species isolated from *banh men*, a fermentation starter from Vietnam is *S. fibuligera* that has the ability to hydrolyse starch. Gandjar (2003) stated that the moulds in the *ragi* are strong amylolytic and degrade mainly the carbohydrate of the rice or glutinous rice into simple sugars which are then further decomposed by the yeasts into alcoholic compounds.

2.3.1 Fungi

Fungi regarded as filamentous organisms, form dry colonies when growing on agar or natural substrates and their colour is generally depend on the pigmentation of their spores (Wainwright, 1992). Most industrially important fungi are either single-celled yeasts or grow as filaments called hyphae. These usually measure from 0.5 μm to 1.0 mm in diameter and can aggregate to form a mycelium (Deacon, 1984; cited in Wainwright, 1992).